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Hypothesis: Can drug-loaded platelets be used as delivery vehicles for blood-brain barrier penetration?

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ABSTRACT

Neurovascular conditions are disorders associated with the blood vessels of the brain that are extremely difficult to treat successfully due to the selectivity and fastidious nature of the blood- brain barrier. Consequently, the efficacy of the pharmacological treatments for these conditions are greatly reduced thereby resulting in large amounts of neurovascular-related morbidity and mortality. Platelets are an important component of blood that actively respond to neurovascular distress in the body. Recent research has proven the effectiveness of platelets as drug delivery vehicles, during circumstances where the body naturally elicits a platelet response. This hypothesis highlights the theoretical use of platelets as drug delivery vehicles, able to penetrate the blood-brain barrier, for the treatment of two neurovascular conditions; glioblastoma multiforme and ischemic stroke. The success of the hypothesised system may lead to the development of a novel and extremely necessary delivery mechanism.

Introduction

The brain is the control centre of the body and therefore requires efficient protection in the form of the blood-brain barrier. Endothelial cells lining the cerebral vasculature form the blood-brain barrier [1] that serves to separate the extracellular fluid of the brain, from the circulating blood. The high selectivity as well as sensitivity of this permeability barrier makes drug delivery to the brain tissue difficult to achieve [2], and as a result the clinical failure of many potentially effective central nervous system therapeutics are often associated with shortcomings in their methodology of drug delivery, and not as a result of a lack in the drugs pharmacological efficacy. Majority of the existing therapeutics, which are available on the market, are rendered ineffective in the treatment of central nervous system diseases, due to the fact that they cannot be delivered and sustained within the brain effectively. This has led to an increase in the need for invasive procedures to treat central nervous system diseases, or alternatively, an increase in the mortality and morbidity from fatal and debilitating CNS conditions respectively [3]. This is evident from the following statistics: 6.5 million deaths occurred due to strokes in 2013, making strokes the second highest cause of death, globally [4]. Brain tumours are the most common cancer, and the leading cause of cancer-related deaths in children aged 0–14 years [5], while brain and central nervous system tumours are the third most common causes of cancer related deaths among the age group of 15–39 years [5].

Vascular disease refers to a class of disorders affecting the blood vessels of the body that results in a range of disorders, which may be severe and even fatal [6]. Gliomas and ischemic strokes are two examples of neurovascular disorders [7]. Glioblastoma multiforme is the most aggressive and the most common type of primary brain tumours [8] that have gained attention due to their complex character which gives them the ability to evade various therapies. The majority of glioblastoma multiforme sufferers die of the disease within a year of diagnosis, and almost none of the patients experience long term survival [9]. Due to the infiltrating nature of these gliomas, the current treatment involves the surgical resection of as much of the tumour as is considered safe, followed by treating the remainder of the tumour via chemotherapy and radiotherapy [10]. However, mortality levels still remain high due to the presence of the blood-brain barrier, as well as the resistance to radiotherapy of the tumour cells present in areas of hypoxia [11].

A stroke is a vascular condition in which brain cell death occurs within minutes due to a reduction in oxygen to areas of the brain [12]. Atherosclerosis, can cause an interruption of the blood flow to an area of the brain resulting in the development of a stroke, whereas a brief interruption/disturbance in the blood supply to the brain can result in the occurrence of a transient ischemic stroke, which places patients at a greater risk of developing more serious strokes [13]. The most common

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methods of treatment for ischemic strokes include medications such as aspirin, and an IV injection of a tissue plasminogen activator [14] that have proven to be effective if they are given within 3–4 h of the detection of the clot, however these medications are entering the systemic circulation and will therefore have a myriad of side effects [15]. Oral medication undergoes first pass hepatic metabolism, indicating that their onset of action is slower compared to other methods of drug delivery, and a smaller amount of the drug will reach the site of action [16].

Platelets, tiny cell fragments of megakaryocytes, are an important component of blood that cross the blood–brain barrier easily during times of vascular disturbance [17]. Numerous platelets are produced in the bone marrow [18] on a daily basis (approximately 2×10^{11} to 5×10^{11}), with the number rising as the need of the body increases [19]. Before being removed by reticuloendothelial cells in the liver and spleen, the platelet lives on average for 7–10 days *in vivo*. Platelets are continuously being researched as promising strategies to achieve supreme systems of therapeutic intervention as they are used as devices that allow for precise and targeted therapy, therefore leading to minimal interaction with non-target cells, which reduces the likelihood of adverse effects occurring. The majority of the platelet-based therapeutic systems, which include the use of natural platelets acting as drug delivery vehicles, biomimetic systems based on platelet membranes, and genetically engineered or hybrid platelet membranes, take advantage of the natural pathophysiological affinity and the ability of platelets to target sites of vascular distress [20].

Hypothesis

A number of studies have shown that platelets are able to act as a drug delivery vehicle by encapsulating drugs effectively. Their aggregation and activation occurs during times of vascular distress in the body. Therefore, this hypothesis proposes the use of platelets as a drug delivery vehicle, able to penetrate the blood-brain barrier, for use in the treatment of glioblastoma multiforme, as well as ischemic stroke. The primary aim of the platelet system is to achieve delivery of the necessary drugs directly to the sites of action. The proposed delivery system, which may be administered via IV injection, takes advantage of the innate function of platelet activation, in order to deliver drugs to the affected areas of the brain. The formulation can be prepared by encapsulating drugs into the platelet canalicular system. The success of this system may result in the achievement of targeted drug delivery, a decrease in systemic side effects, and a great reduction in the time to the pharmacological onset of action of the drug. The ultimate goal of the hypothesised treatment paradigm is a decline in the morbidity and mortality associated with the abovementioned neurovascular conditions.

Evaluation of the hypothesis

Platelet drug delivery vehicles

Platelets are an ideal drug delivery system as they only target the desired affected cells and thereby have a minimal effect on surrounding healthy tissue, causing the incidence of systemic side effects to be greatly reduced. Drug release from platelet carriers occurs in a controlled manner; and good biocompatibility, good biodegradability, and immune evasion is ensured [21]. Platelets have been found to have the ability to encapsulate drugs through their canalicular system [22], and when platelet activation occurs, granular matter as well as the encapsulated drug are released from the platelet [22]. Therefore the success of this drug delivery system is dependent on platelet aggregation and subsequent activation. Drug entrapment by platelets occurs during exposure of the platelet to the drug, and this entrapment occurs at a relatively high concentration [23]. When platelets encapsulate drugs, they cloak and protect the drug in a manner where the body does not detect it as foreign matter and therefore does not cause increased clearance of these platelets [24]. This leads to a prolonged circulation in the blood, thereby increasing the concentration of the drug in the body and decreasing the clearance rate of the drug from the body. The innate function of platelets is to target vascular disorders, such as, but not limited to, cancer, thrombosis, haemorrhage and strokes [7], leading to an increasing interest in using platelets, as a drug delivery vehicle for the purpose of treating these disorders [25]. Being a biodegradable drug delivery system means that the platelet carrier system will not have to be removed from the body once the treatment is complete.

The application of platelets as delivery vehicles for the treatment of glioblastoma multiforme

Cancer cells are able to interact with platelets when the cells enter into blood vessels, with the aim of causing the release of platelet factors that will increase, as well as improve cancer cell vascular permeability, and promote motility and survival of the cells, thereby aiding cancer cell growth and metastasis [26]. The process whereby tumour cells and platelets interact is called tumour cell-induced platelet aggregation (TCIPA) [27], which involves the generation of receptor cells on the surfaces of the tumour cells that allow for platelet aggregation [26]. As has been mentioned before, platelet aggregation and subsequent activation is necessary for the effective functioning of the platelet drug delivery system. Following the process of TCIPA, platelet cells are activated and subsequently release factors such as factor VIIa, serine proteases, factor Xa, fibrinogen, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and thrombospondin [26]. As these factors are released from the platelets, the encapsulated drug within the platelet carrier is released as well [22].

Studies conducted by Xu et al. [22], Sarkar et al. [28], and other researchers, proved that the chemotherapeutic drug, doxorubicin, was effectively encapsulated into natural platelets. Their findings proved that these drug loaded platelets still maintained their morphology and integrity and were therefore still detected by the body as natural, unloaded platelets. We postulate that this finding ensures that these doxorubicin-loaded platelets would still be able to cross the blood-brain barrier without being detected as foreign matter. The studies also found that the drug loaded platelets were successful in significantly inhibiting tumour growth, all the while ensuring immune system evasion and biocompatibility [22,28]. Doxorubicin has been proven to be an effective drug against glioblastoma multiforme [29], which indicates that doxorubicin-loaded platelets can be used as a post-surgical treatment modality, allowing for targeted drug release, directly to the tumour sites unable to be surgically resected. Consequently, decreasing the harmful systemic side effects of the chemotherapeutic drug, which is problematic for the majority of patients, and ensuring a better chance of positive treatment outcomes.

The application of platelets as delivery vehicles for the treatment of ischemic strokes

In the process of genesis of an ischemic stroke, platelets aggregate to form a blood clot and send out signalling molecules which act to initiate subsequent platelet arrival to the site of the clot [30]. Following platelet arrival to the site, as part of the clotting cascade process, the platelets undergo aggregation as well as activation to enhance clot formation [31]. We hypothesize using platelets as a drug carrier for an effective and appropriate anticoagulant drug. The platelet drug carriers will encapsulate and cloak the drug, and transport it through the blood-brain barrier, directly to the ischaemic site. When the platelet becomes activated, by being exposed to the clot, it will release its granular contents as well as the drug, in a similar manner as has been described above. The patient will not have to undergo any medical procedure, the treatment time will be much shorter, the incidence of positive

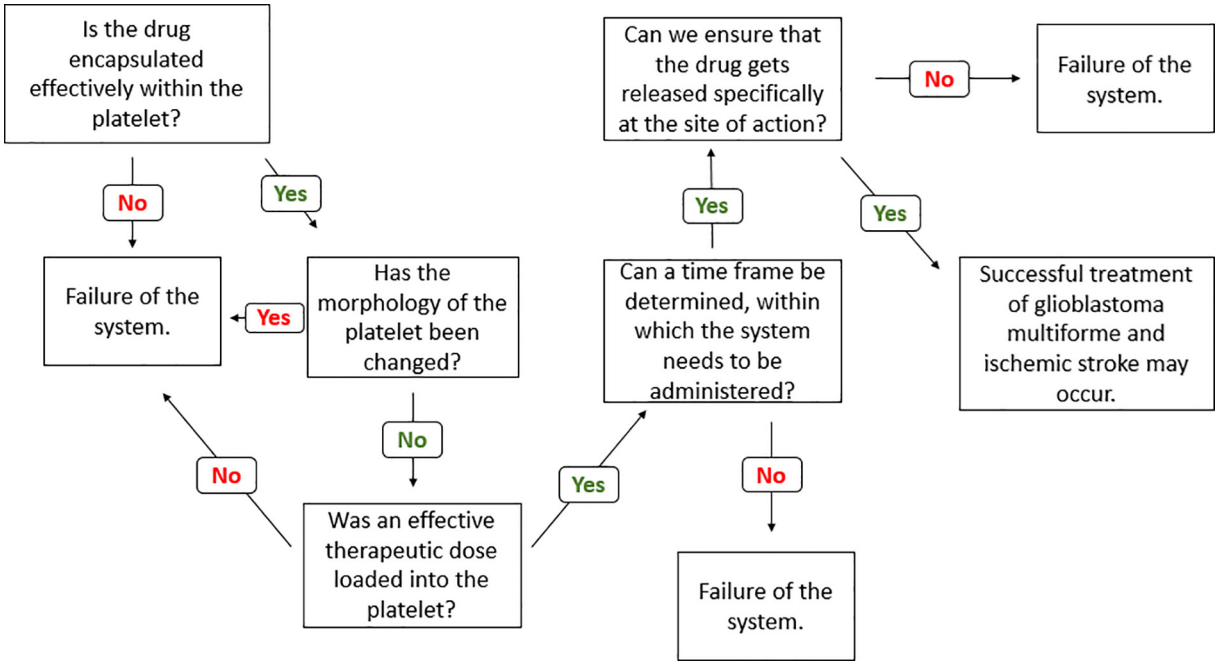

Fig. 1. Schematic detailing the first 4 inquiries and consequences of the platelet drug delivery system, intended to cross the blood-brain barrier.

treatment outcomes will be greater, and there will be less complications due to drug therapy, thereby increasing the likelihood of successful treatment. Fig. 1 is a schematic representation of the mechanism of operation of the hypothesized platelet drug delivery system.

The stability of platelet drug delivery vehicles

In the study conducted by Xu and co-workers [22] the stability and aggregation behaviour of the doxorubicin-loaded platelets were compared with that of natural, unloaded platelets, by tests performed at different time points. The results indicated that the loss of functionality of the doxorubicin-loaded platelets and the unloaded platelets were similar and occurred in a time-dependent manner. This finding proves the stability of the doxorubicin-loaded platelet system. We have used these results to postulate the stability of the platelet drug delivery system within the context of crossing the blood-brain barrier.

Discussion on the significance of the platelet drug delivery system

Using platelets as drug delivery vehicles that carry medication across the blood-brain barrier, may emanate in a large improvement, as well as enhancement in the treatment of glioblastoma multiforme and ischemic stroke. The hypothesis may be tested by carrying out the necessary experimentation in the laboratory to determine the effects of the drug and platelet conjugation, as well as the formulations' ability to cross the blood brain-barrier successfully. I*n vitro*, *ex vivo* and *in vivo* stability testing of the developed formulation may be performed in order to determine the safety, efficacy and drug release behaviour of the formulations [32]. Recent experimentation has shown a wide array of advantages in employing red blood cells using hitchhiking mechanisms to deliver nanoparticles [33]; we therefore further hypothesise the use of the platelet-based drug delivery system as a carrier for nanoparticle hitchhikers that may cross the blood-brain barrier with ease. The implications of the success of this overall hypothesis will be the development of a much needed drug delivery system, able to easily penetrate the blood-brain barrier during times of vascular distress. This approach to treatment may lead to the formation of a novel advancement in the treatment of glioblastoma multiforme, ischemic strokes and all neurovascular conditions that elicit platelet activation, leading to a decrease in the mortality and morbidity associated with these conditions, and thereby enhancing and improving the quality of life of the affected patients worldwide. The hypothesised delivery system is not applicable to synthetic platelet-like particles as there is a great obstacle in ensuring that the synthetic structures created duplicate the complex platelet make-up with great accuracy. This entire system is dependent on the body recognising the vehicles as natural platelets in order to avoid the occurrence of immunogenicity, as well as allowing the platelets to penetrate the blood-brain barrier, therefore this hypothesis excludes synthetic platelet-like particles [34].

Conclusions and implications of the hypothesis

This hypothesis raises a number of key inquiries such as, (1) when would the platelet formulation need to be administered to the patient suffering from an ischemic stroke? (2) How can it be ensured that the drug is released specifically in the brain and not at another site of the body that may simultaneously elicit a platelet response? (3) Is it possible to attain and control effective dosing, based on the amount of drug that can be encapsulated within a platelet vehicle? (4) Could the anticoagulant drug change the morphology of the platelet, and thereby cause the blood-brain barrier to deem the platelet as a foreign substance? (5) Is it possible to control drug release from the platelet carrier, by investigating the exact chemical mechanism of entrapment of drugs within the platelet?

The first inquiry can be deduced by performing haematological tests to determine the time frame in which additional platelets will still be signalled to the site of the clot in the brain, thereby giving an indication of the duration in which this system needs to be administered in order to perform the function effectively. The second inquiry can be addressed by the process of selective brain targeting using a ligand [35]. A specific ligand may be chosen that can form a complex with a biomolecule on the surface of the tumour in glioblastoma multiforme, and a biomolecule at the site of the clot in an ischemic stroke, ensuring that the platelet vehicle travels to the brain and subsequent release of the drug will occur specifically at the affected area of the brain only. Previous research has not shown any negative results relating to inquiries 3 and 4, however research has never been done on the conditions mentioned in this hypothesis. Therefore, beyond extrapolation, laboratory

testing will be essential to determine the outcome of inquiries 3 and 4; as specific analytical tests, such as microscopy and western blot tests, are required to make these necessary deductions. Computational biological analyses may be used to determine the mechanism by which drug entrapment occurs within the platelet carrier [36], and the results obtained from these assessments may be used to investigate whether drug release from the platelet can be controlled effectively, in order to address inquiry number 5.

It can be concluded that novel drug loaded platelets is an ideal, theoretical approach for drug delivery across the blood-brain barrier in order to greatly improve the treatment outcomes of glioblastoma multiforme and ischemic stroke.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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